PATENT
Customer Number 22,852
Attorney Docket No. 06478.1452-00

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	
METZNER and SCHNEIDER	Group Art Unit: Unassigned
Serial No.: To be assigned	Examiner: Unassigned
Filed: Concurrently herewith	
For: THROMBIN PREPARATIONS AND PROCESS FOR THEIR PRODUCTION	

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

PRELIMINARY AMENDMENT

Prior to the examination of the above application, please make the following amendments:

IN THE SPECIFICATION:

Please replace the paragraph at page 8, lines 6-14, with the following:

Initially the thrombin solution was mixed with 0.6 mol/l sodium sulfate and adsorbed onto a hydrophobic interaction chromatography (HIC) gel (in this case: Phenyl-Sepharose HP, manufacturer: Amersham Pharmacia, Freiburg, Germany) which had previously been equilibrated with buffer A (10 mmol/l Na phosphate pH 6.5) containing 0.6 mol/l sodium sulfate. After washing with buffer A containing 0.6 mol/l sodium sulfate, the bound thrombin was eluted by a gradient with decreasing sodium

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sulfate content in buffer A. Impurities and thrombin fragments were to a large extent removed in the flow-through or in the wash fractions.

Please replace the paragraph at page 8, lines 15-26, with the following:

The thrombin fraction was loaded without further treatment directly onto a cation exchange column (CEC; in this case: FRACTOGEL® EMD SO_3, manufacturer: Merck, Darmstadt, Germany) equilibrated with buffer A, washed with equilibration buffer A and eluted by a gradient from 0 to 1.0 mol/l sodium chloride in buffer A. During the separation, final byproducts and thrombin fragments were removed so that the resulting α-thrombin eluate had a high specific purity of about 3500 IU/mg (protein determination by determining the absorption at 280 nm and using the conversion factor of 1.74 for a 0.1% strength solution in accordance with J.W. Fenton, II, M.J. Fasco, A.B. Stackrow, D.L. Aronson, A.M. Young and J.S. Finlayson, Human Thrombins. J Biol Chem 252; 3587-3598 (1977)). Table 1 shows the results of this thrombin purification and the resulting specific activity.

Please replace the paragraph at page 9, lines 3-14, with the following:

Starting from a thrombin concentrate of moderate or low purity, two chromatography steps were carried out. Initially the thrombin solution was mixed with 0.6 mol/l sodium sulfate and adsorbed onto a hydrophobic interaction chromatography (HIC) gel (in this case: Phenyl-Sepharose HP, manufacturer: Amersham Pharmacia, Freiburg, Germany) which had previously been equilibrated with buffer B (10 mmol/l Na phosphate 0.1% PEG pH 6.5; (in this case PEG 6000, but other molecular weight

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ranges can also be employed)) containing 0.6 mol/l sodium sulfate. After washing with buffer B containing 0.6 mol/l sodium sulfate, the bound thrombin was eluted by a gradient with decreasing sodium sulfate content in buffer B. Impurities and thrombin fragments were to a large extent removed in the flow-through or in the wash fractions.

Please replace the paragraph at page 9, lines 15-19, and page 10, lines 1-3, with the following:

The thrombin fraction was loaded without further treatment directly onto a cation exchange column (CEC; in this case: FRACTOGEL® EMD SO_3, manufacturer: Merck, Darmstadt, Germany) equilibrated with buffer C (10 mmol/Na phosphate, 166 mmol/l L-arginine pH 6.5), washed with equilibration buffer C and eluted by a gradient from 0 to 1.0 mol/l sodium chloride in buffer C. During the separation, final byproducts and thrombin fragments were removed so that the resulting α-thrombin eluate had a high specific purity of about 3300 IU/mg (cf. Table 2).

Please replace the paragraph at page 10, lines 15-16, and page 11, lines 1-7, with the following:

Starting from a thrombin eluate purified as in Examples 1 to 3 and after hydrophobic interaction chromatography and cation exchange chromatography, a filtration was carried out on a membrane with a small pore size (e.g. PLANOVA™ 15 nm). Even small viruses such as parvoviruses can be effectively removed with this membrane. It was found that on use of the purified thrombin as starting material, very good yields in terms of thrombin activity and protein were obtained, with a good filtration

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rate (see Table 3). This process is therefore suitable for producing a thrombin concentrate with high virus reduction factors.

Please delete text "AVENTIS BEHRING GMBH 2000/A002-A1" at page 16, line 1.

Please replace text at page 16, line 2, with the following:

We claim:

IN THE CLAIMS:

Please cancel claims 1-17, and add new claims 18-34, as follows:

18. (NEW) A thrombin preparation comprising a noncovalently binding inhibitor of thrombin activity as stabilizer.

19. (NEW) The thrombin preparation as claimed in claim 18, which additionally comprises a soluble calcium salt and sodium chloride as stabilizers, a buffer substance, and further comprises at least one of

a sugar,

a sugar alcohol,

an amino acid,

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a salt of a mono- or polycarboxylic acid, or

a salt of a mono- or polyhydroxycarboxylic acid,

wherein the thrombin preparation is stable in the liquid state.

20. (NEW) A process for producing a thrombin preparation, comprising a prothrombin obtained from plasma or a plasma fraction, wherein, following activation of the prothrombin to thrombin, and optionally further processing steps, the thrombin is purified by hydrophobic interaction chromatography.

21. (NEW) The process as claimed in claim 20, wherein the prothrombin employed for activation to thrombin is subjected to inactivation or reduction of viruses during its production.

22. (NEW) The process as claimed in claim 20, wherein the thrombin is subjected to inactivation or reduction of viruses before or after hydrophobic interaction chromatography.

23. (NEW) The process as claimed in claim 20, additionally comprising cation exchange chromatography carried out before or after the hydrophobic interaction chromatography.

24. (NEW) The process as claimed in claim 20, wherein the thrombin preparation is adjusted to a pH of from 5.0 to 8.0.

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25. (NEW) The process as claimed in claim 20, wherein a soluble calcium salt and sodium chloride as stabilizers, a buffer substance, and at least one of

a sugar,

a sugar alcohol,

an amino acid,

a salt of a mono- or polycarboxylic acid, or

a salt of a mono- or polyhydroxycarboxylic acid,

are added to the thrombin preparation.

26. (NEW) The process as claimed in claim 20, wherein a noncovalently binding inhibitor of thrombin activity is added as a stabilizer.

27. (NEW) The process as claimed in claim 26, wherein the noncovalently binding inhibitor of thrombin activity is benzamidine or p-aminobenzamidine.

28. (NEW) The process as claimed in claim 20, wherein a gel with coupled hydrophobic radicals is employed as absorbent for the hydrophobic interaction chromatography.

29. (NEW) The process as claimed in claim 28, wherein the hydrophobic radicals of the gel employed as absorbent are phenyl radicals or ligands of similar hydrophobicity.

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30. (NEW) The process as claimed in claim 20, additionally comprising filtration of the thrombin preparation through a membrane with a suitable pore size to remove viruses.

31. (NEW) A thrombin preparation, which is obtainable by the process of claim 20.

32. (NEW) A method of using the thrombin preparation of claim 18 as a hemostatic, a constituent of a hemostatic or as a constituent of tissue glue.

33. (NEW) A method of using the thrombin preparation of claim 19 as a hemostatic, a constituent of a hemostatic or as a constituent of tissue glue.

34. (NEW) A method of using the thrombin preparation of claim 31 as a hemostatic, a constituent of a hemostatic or as a constituent of tissue glue.

REMARKS

Claims 1-17 have been cancelled. New claims 18-34 have been added and correspond to claims 1-17 as filed. Applicants submit that these amendments conform the claims to United States Patent and Trademark Office practice and, as such, introduce no new matter.

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Several paragraphs of the specification have been replaced in order to correct informalities and place trademark names in proper United States Patent and Trademark Office format.

If there is any fee due in connection with the filing of this Preliminary Amendment, please charge the fee to our Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.

Dated: March 15, 2001

By: Carol P. Einaudi

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APPENDIX TO PRELIMINARY AMENDMENT

AMENDMENTS TO THE SPECIFICATION

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Please replace the paragraph at page 8, lines 15-26, with the following:

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Please delete the paragraph at page 16, line 1 as follows:

[AVENTIS BEHRING GMBH 2000/A002-A1]

Please replace the paragraph at page 16, line 2, with the following:

[Patent claims for the USA] We claim:

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